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PHYTOCHEMICAL ANALYSIS OF LEAF EXTRACT OF *Calliandra haematocephala* AND IN VITRO ANTIBACTERIAL ACTIVITY AGAINST FOOD BORNE BACTERIA

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Food poisoning is the effect of eating toxic, contaminated or spoiled food. An estimated 600 million people in the world fall ill after eating contaminated food and 420000 die every year. Most of the food contamination occurs due to bacteria. They can survive in food at any stage i.e. when food is growing, packaged, shipped or cooked. Prohibition of food spoilage is accomplished by use of chemicals, but they have side effects on human health. Attempts have been targeted in the production of natural preservatives which are nutritionally safe and ensure quality of food products. Air dried powder of leaves of *C. haematocephala* was extracted in soxhlet apparatus with 80% ethanol. *S. aureus, E. coli, S.typhi, P. aeruginosa* and *B. subtilis* were isolated and identified biochemically. Antibacterial activity of plant extract was determined against these bacteria. MIC and MBC were also determined. Phytochemical analysis was done using high performance liquid chromatography (HPLC). Disc diffusion method showed zones of inhibition of 13, 15, 11, 14 and 12mm against *E. coli, S. aureus, P. aeruginosa, B. subtilis* and *S. typhi*, respectively. MIC for *E. coli, S. aureus, P. aeruginosa, B. subtilis* and *S. typhi* was 312, 78, 25×10^2, 156 and 312µg per 50µl, respectively. Phytochemical analysis showed that flavonoids and phenolics were present in the extract. Ethanolic extract of *C. haematocephala* have active compounds that can be used as natural additive in food.
Keywords: Food poisoning, foodborne sickness, diarrheal diseases, food additives, ethanolic extract, antimicrobial agent.

INTRODUCTION

Food poisoning is a group of disease caused by consumption of contaminated food with variety of factors ranging from infectious organisms or their toxins to both organic and metallic chemical contaminants. Foodborne sickness consists of broad spectrum of diseases and are responsible for significant mortality and morbidity globally. Food borne diseases are the major health issue in both developed as well as developing countries. More than 250 food borne diseases are caused by different pathogens. Bacteria are the causative agents of two third of food borne disease outbreak (Hayajneh, 2015). An estimated 600 million – almost 1 in 10 people in the world fall ill after eating contaminated food and 420000 die every year, resulting in the loss of 33 million healthy life years (DALYs). Children under 5 years of age carry 40% of the foodborne disease burden, with 125 000 deaths every year. Diarrheal diseases are the most common illnesses resulting from the consumption of contaminated food, causing 550 million people to fall ill and 230000 deaths every year (WHO, 2018).

Food additives are substances added intentionally to foodstuff, to increase the durability of the product and enhance or modify its properties, including its appearance, flavor or structure, provided it does not distract from its nutritional value. Preservatives are food additives that protect against the action of microorganisms (fungi and/or bacteria) and thereby extend the shelf life of foodstuff. Prohibition of food contamination and their biological agents are usually attained using chemical preservatives. Although the efficacy of these synthetic preservatives has been proven in inhibition of food borne illness, but their repeated use results in the aggregation of chemical remains in the food web, microbial resistance and side effects on human health (Javed, 2016). So, there is need to produce natural, healthy and effective food preservatives. These plant extracts are regarded as natural antimicrobial agents which are nutritionally safe and ensure quality of food products. Plant extracts increase the shelf life of food and enhance nutritional properties. Due to these natural preservatives there will be minimum processing requirements (Sharif *et al.*, 2017).

Calliandra haematocephala (red powder puff) belonging to a family Fabaceae. It is an ornamental plant. It is a sprawling well branched evergreen shrub. Studies shows that red powder puff has anti-inflammatory, anticonvulsant, antiulcerogenic, gastro protective and immunoadjuvant properties. The decoction of the flower extract is used as blood purifier and tonic everywhere because of its antioxidant property. Its roots are used for hemorrhoids. It is reported that betulinic acid in plant is responsible for its antitumor, anti-HIV and anti-rotaviral activity. It is used for hepatoprotection and has antioxidant activity, due to the free radical scavenging effect by the phenolics, saponins and flavonoid contents of the extract (de Paula Barbosa, 2014).

Phytochemical analysis of leaves extract of *C. haematocephala* shows that it consists of carbohydrate, proteins, steroids, flavonoids, glycosides, phenolics, saponins, alkaloids, tannins and fats. Both tannins and flavonoids exhibits greater antimicrobial activity (Gupta *et al.*, 2013).

C. haematocephala conventionally used as antimicrobial agent. Antimicrobial and anti-helminthic activity has been observed by its flower extracts. Its bark contains compounds with antimicrobial activity. Silver nanoparticles manufactured from leaf extract of *C. haematocephala* also used against microbes (Raja *et al.*, 2017). Leaves extract of *C. haematocephala* also has good antimicrobial activity against different bacterial strains specifically by phenolics, flavonoids and tannins in the extract. Methanolic extract *C. haematocephala* leaves has good antiviral activity (Punnagai and Muthiah, 2017). Similarly, leaves extract exhibited antifungal activity particularly by certain nonprotein amino acids present in *C. haematocephala* (Brenner and Romeo, 1986).

MATERIALS AND METHODS

***Plant extract preparation*:** Leaves of *C. haematocephala* were collected from 32 square, University of Agriculture Faisalabad and recognition and authentication was done from Botany Department, University of agriculture Faisalabad. Leaves were air dried and crushed to powdered form. 20 grams of air-dried powder of leaves of *C. haematocephala* was extracted in Soxhlet apparatus with 250 ml of 80% ethanol. Excess solvent was removed using rotary evaporator. The final extract was conserved in airtight container at 4°C in refrigerator till further use.

***Sample processing*:** Food samples rice, chicken, milk, fruits and vegetables were processed in normal saline and inoculated on nutrient agar, MacConkey agar, Mannitol salt agar, Salmonella shigella agar and cetrimide agar and incubated at 37°C for 24 hours.

***Antimicrobial susceptibility testing*:** The antibacterial activity of ethanolic extract of *C. haematocephala* leaves were determined against *S. aureus, P. aeruginosa, E. coli, S. typhi and B. subtilis* by disc diffusion method.

***Minimum inhibitory concentration (MIC)*:** MIC of plant extract was performed in micro dilution plate. 50 µl of extract with two-fold serial dilution and 50 µl of nutrient broth was added in micro dilution plate up to well no. 12. Then 20 µl of bacterial inoculums was added up till well no. 12. Well no. 1 and 2 were maintained as control negative and positive containing nutrient broth + bacterial inoculum and extract + nutrient broth, respectively. After incubation of 24 hours, results were noted. The turbidity was closely observed.

***Minimum bactericidal concentration (MBC)*:** To check the MBC, wells showing no visible growth in MIC, 4ul of mixture were transferred onto fresh nutrient agar plate with micropipette by spreading method and incubated at 37ºC for 24 hours.

***HPLC*:** Phytochemical analysis of ethanolic abstract of *C. haematocephala* was done by high performance liquid chromatography (HPLC) at central Hi-tech Lab., University of agriculture, Faisalabad. High performance liquid chromatography (HPLC) by adding the plant samples in HPLC grade methanol at 0.1mg/µl concentration and strained through 0.2 millipore membrane filter. It was then subjected on RP-18 column. The fractions correlating to maximum peaks with fixed retention time were collected by using a fraction collector. HPL Chromatography analysis was performed using two LC-10AT pumps (Shimadzu).

RESULTS

***Bacteria isolation*:** *S. aureus, P. aeruginosa, E. coli, S. typhi and B. subtilis* were then recognized by their colony morphology, gram staining and biochemical test including catalase test, citrate test, coagulase test, methyl red, oxidase test and TSI test utilizing the standard methodology for identification (Table 1).

The extracts of *C. haematocephala* was worked out by disc diffusion method. It is compared with positive control which

Table 1. Results for minimum inhibitory concentrations and minimum bactericidal concentrations of *Callandra haematocephala* extracts against different bacteria

Well No.		1	2	3	4	5	6	7	8	9	10	11	12
Dilutions of plant extract		__	__	1/1	½	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512
plant extract ug per 50ul		__	__	5×10^3	25×10^2	12×10^2	625	312	156	78	39	19.5	9.7
S. aureus	MIC	✓	×	×	×	×	×	×	×	×	✓	✓	✓
	MBC	✓	×	×	×	×	×	×	×	✓	✓	✓	✓
E. coli	MIC	✓	×	×	×	×	×	×	✓	✓	✓	✓	✓
	MBC		×	×	×	×	×	✓	✓	✓	✓	✓	✓
P. aeruginosa	MIC	✓	×	×	×	✓	✓	✓	✓	✓	✓	✓	✓
	MBC	✓	×	×	✓	✓	✓		✓	✓	✓	✓	✓
S. typhi	MIC	✓	×	×	×	×	×	×	✓	✓	✓	✓	✓
	MBC	✓	×	×	×	×	×	✓	✓	✓	✓	✓	✓
B. subtilis	MIC	✓	×	×	×	×	×	×	×	✓	✓	✓	✓
	MBC	✓	×	×	×	×	×	×	✓	✓	✓	✓	✓

Table 2. Different phenolics and flavonoids identified through HPLC from the extracts of *Callandra haematocephala*

	Compounds Name	Retention time	Quantity in ppm	Area (mV.s)	Area (%)
Standard	T-Butanol	2.853		150.843	10.2
Flavonoid	Quercetin	3.273	3.43	64.828	4.4
	Gallic acid	4.453	6.12	167.256	3.1
	Caffeic acid	12.376	1.12	24.495	1.7
	Vanillic acid	13.747	0.74	12.005	0.8
Phenolics	Chlorogenic acid	15.287	0.21	2.877	0.2
	Syringic acid	16.253	1.38	55.275	3.8
	P-coumeric acid	17.567	0.35	27.302	1.9
	Cinamic acid	25.167	3.63	103.997	7.1
	Sinapic acid	26.633	1.27	98.273	6.7

are antibiotic. Ampicillin is used for *S. aureus*, *E. coli* and *S. typhi*. Streptomycin was used for *B. subtilis* and gentamycin was used for *P. aeruginosa*. Disc diffusion method showed zones of inhibition of 13, 15, 11, 14 and 12mm against *E. coli*, *S. aureus*, *P. aeruginosa*, *B. subtilis* and *S. typhi*, respectively (Table 1).

***MIC and MBC*:** Table 1 shows the results of MIC of plant extract performed in micro dilution plate. The turbidity in row containing *S. aureus* was observed in well no. 10, the turbidity in row having *S. typhi* was noted in well no. 8, the turbidity in row containing *E. coli* was observed in well no. 8, row containing *B. subtilis* was observed in well no. 9 and similarly row having *P. aeruginosa* was observed in well no. 4. Similarly, the results for MBC on petri plates are observed after incubation and plate with no visible growth prior to growth on plate are considered as MBC value.

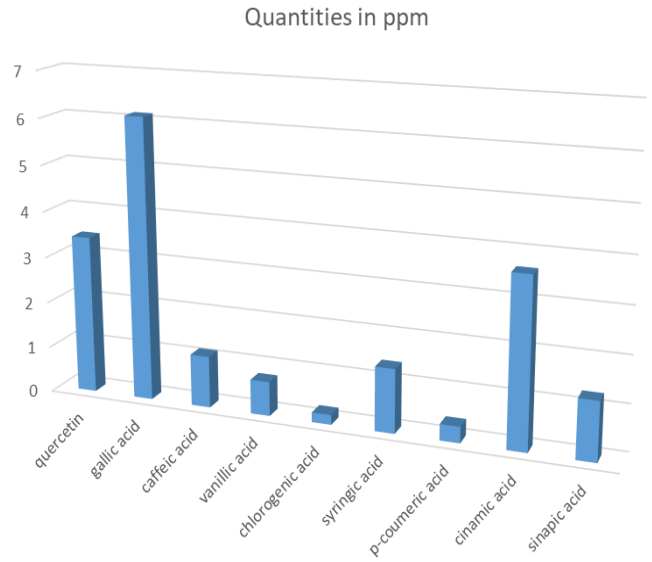

Figure 1. Quantitative analysis of different phenoloics and flavonoids extractetd from *Callandra haematocephala*.

***HPLC analysis*:** The extracts of *Callandra haematocephala* were subjected to HPLC as shown in Table 2 and Figure 1. A wide variety of phenolics and flavonoids was screened from the extracts of *Callandra haematocephala* with a wide range quantitatively.

DISCUSSION

Food poisoning caused by consumption of contaminated food. Most of the food poisoning cases are linked with bacterial contamination. Prohibition of food contamination and their biological agents are usually attained using chemical preservatives, but they have side effects on human health. Attempts have been targeted in production of natural preservatives. These plant extracts are regarded as nutritionally safe and ensure quality of food products.

Literature exhibited the preparation of extract of *C. haematocephala* using conventional method utilizing ether as solvent, this extract showed antimicrobial activity against 3 bacteria but no activity against *P. aeruginosa*. In the present study the extraction method used for the preparation of our extract was Soxhlet apparatus utilizing ethanol solvent. It works on the principle of boiling and condensation. The extract obtained showed good antibacterial activity against all tested strains including *P. aeruginosa* which showed that extract obtained from this method was more effective and it was less time consuming because extract was obtained in 8 hours.

The literature explained that that the ether extract of *C. haematocephala* showed good antimicrobial activity against *S. aureus*, *B. subtilis* and *E. coli*. At concentration 3.9 µg/ml the extract produced zone of inhibition against *S. aureus* (22mm), *E. coli* (20mm) and *B. subtilis* (24mm) and exhibited MIC values of 0.12, 0.007 and 0.49 µg/ml against *S. aureus*. *B. subtilis* and *E. coli*, respectively. In this study the extract showed no antibacterial activity against *P. aeruginosa* (El-Sayed and El-ghaly, 2014). In present study, antibacterial activity of the ethanolic extract of *C. haematocephala* was checked against five food borne bacteria i.e. *E. coli*, *S. aureus*, *S. typhi*, *B. subtilis* and *P. aeruginosa* and the extract

exhibited good antibacterial activity against all tested organisms. The extract showed maximum activity against *S. aureus* and *B. subtilis* and then against *S. typhi* and least against *E. coli* and *P. aeruginosa*.

Conclusion: In previous studies HPLC of extract of *C. haematocephala* was done to check active chemicals which were usually phytoconstituents. One study exhibited the identification of different compounds which were carbohydrates, fats or terpenoids in nature. The compounds include β-sistosterol, dodecanoic acid and lupeol. Another study exhibited the determination of protein and carbohydrate contents of abstract of *C. haematocephala*. Total phenolics and flavonoids contents of alcoholic extract were done by Folin–Ciocalteu in one of the studies. Literature also showed the identification of tannins, alkaloids, saponins and flavonoids from the extract of *C. haematocephala*. Different types of flavonoids like quercetin and condensed tannins were determined in another study. In present study, flavonoids and phenolic were isolated from ethanolic extract of leaves of *C. haematocephala.* Different phytochemicals of class flavonoid and phenolics including Quercetin, Gallic acid, Caffeic acid, Sinapic acid and many more were identified using HPLC technique. So present study shows that leaves extract of *C. haematocephala* exhibited antibacterial activity against tested food borne bacteria and good inhibition zones. So, this extract can be used as natural food supplement in food industry.

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